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Abnormalities in the Ultrastructure of Melanocytes and the Outer Root Sheath of Clinically Normal Hair Follicles from Alopecia Areata Scalps

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A subclinical condition of alopecia areata has been described [1], in which abnormalities seen in follicles from active alopecia areata were also found to some extent in follicles from clinically normal, that is non-balding, regions of alopecia areata scalps. Ultrastructural examination of the hair follicles from non-balding regions of alopecia areata may reveal important information about the changes involved and help indicate the etiology of the disease.

Our previous investigations have identified abnormalities in the orientation of the cells of the dermal papilla [1] and evidence of ultrastructural abnormalities‡§ within the cells and of the dermal papilla–epithelial junction in follicles from clinically normal areas of alopecia areata scalps. Melanocytes have been implicated in the etiology of alopecia areata and regrowing hairs are often white [2]; because abnormalities of melanocytes and an unusual outer root sheath distribution have been reported [3] we have extended our investigations to the melanocytes and epithelial components of the clinically normal follicle.

We have compared the ultrastructure of clinically normal hair follicles with those of normal control scalps and active lesional edges to determine the earliest changes at the ultrastructural level that may indicate the primary site of damage in the alopecia areata follicle. Scalp biopsies (4 mm) were taken from five normal controls and from six alopecia areata patients on first presentation. Patient biopsies were taken from the "active" edge of a patch of alopecia areata and a non-balding area within the same scalp bearing clinically normal terminal hair. Control and non-balding region biopsies were taken from the occipito-parietal region. Individual follicles were microdissected, processed routinely for electron microscopy, and sectioned longitudinally through the middle plane of the follicle [4].

We have examined the ultrastructure of the hair bulb adjacent to the upper regions of the dermal papilla and found that all follicles contained melanocytes in the undifferentiated matrix and presumptive cortical regions. Normal follicles always had well developed melanocytes with melanosomes actively engaged in pigment transfer. In follicles from active alopecia areata regions melanosomes were generally absent or were small and poorly differentiated. Follicles from non-balding regions either contained well-formed melanocytes that were actively engaged in pigment transfer when there were few or no other signs of cellular degeneration or, conversely, showed varying degrees of abnormality of melanocyte distribution and morphology.

We have not seen the unusual presence of melanocytes in the outer root sheaths as described by Tobin *et al* [3]
areata, but we have noted other degenerative tendencies that correspond well with the hypothesis of a sub-clinical condition in the disease. The cells of the outer root sheath were vacuolated to some extent in all follicles, outermost cells more so than inner. This vacuolation is attributed to loss of glycogen during preparation for microscopy [5]. Non-balding region follicles resembled normal follicles below about half papilla height. However, above half papilla height there was evidence of more extensive vacuolation and most noticeable was the marked presence of a fine granular deposit. In follicles from active lesions of alopecia areata vacuolation was even more pronounced, but no granular deposits were observed.

Overall, degenerative trends in the structure, composition, and activity of melanocytes and melanosomes appeared to be dependent on the degree of activity of the disease. In the outer root sheath of non-balding region follicles cells were deteriorated and granular deposits were seen, whereas the inner epithelial cells appeared to be in quite good condition. The fine granular deposit observed in the follicles is probably glycogen. Small amounts were detected in some normal hair follicles, where it was located mainly on the inner edge of the innermost cells of the outer root sheath. However, in follicles from non-balding regions much larger amounts of granular deposit were distributed over a much greater area of the outer root sheath. A possible explanation for this increased content and

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distribution is that normal differentiation is affected in non-balding region follicles because these quantities of glycogen are seen much higher up in the normal follicle.

At the clinical level, hair in non-balding regions displays the properties of normal hair, but at the ultrastructural level follicles clearly showed properties of active area alopecia follicles. These early changes in the melanocytes and outer root sheath cells could be primary changes in the pathology consistent with the apparent importance of melanocytes in the disease or could be secondary changes due to disturbance or abnormalities of the dermal papilla, which also showed multiple changes. The large cell-surface to volume ratio of melanocytes may well make them exhibit early signs of general distress in the follicle, perhaps due to perturbance of the normal regulatory factors produced by the dermal papilla. Further studies of the sub-clinical condition of alopecia areata may reveal important information about the etiology of the disease.

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Immunity to Hair Follicles in Alopecia Areata

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The cause of alopecia areata (AA) is not known. The favored hypothesis is that it results from an autoimmune response to hair follicles (HFs), based on indirect observations that have recently been reviewed [1].
disease, which may involve hair, eyes, and nails; its association with other autoimmune diseases and autoantibodies to other organs; the presence of a lymphocytic infiltrate around and in hair bulbs during active disease; non-specific alterations in the number and/or function of circulating T cells; deposits of immunoglobulin and complement around HFs particularly at the edge of active lesions; increased expression of class I and II major histocompatibility complex antigens and of Langerhans cells in hair bulbs in active disease; and the fact that effective therapies for AA all have as a common denominator a suppression in the number or function of immune cells in skin. The major problem with this hypothesis is that, until recently, there was no evidence of an abnormal immune response directed specifically to HFs in AA.

We recently have made three significant observations that strongly support the hypothesis that AA is an autoimmune disease. The first is that HFs express unique antigens, and that some of these are the target of autoimmune responses [2].
antigens in HFs was demonstrated by probing Western blots of extracts of isolated human HFs with sera of normal persons. Most individuals had low levels (titer of 20) of antibodies that reacted to multiple antigens in HFs. Many of these antigens were specific for HF and were not expressed in adjacent epidermis or dermis. Unique HF-antigens extractable with NP-40 and 6 M urea had approximate Mw of 84, 105, 115, and 125 kD and 115, 145, 200, and 220 kD, respectively. Most of these antigens were autoantigens, as they reacted with antibodies present in the donor's own serum. The presence of unique antigens in HF that can trigger autoimmune responses provide the framework necessary to explain the selective damage to HF that occurs in AA.

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Second, antibodies to some antigens that are selectively expressed in HFs are present more frequently and in higher levels in patients with AA [3].
detected in all AA patients (100%) but in less than 44% of controls using a Western immunoblot assay [3].
predominantly directed to one or more antigens of approximately 44, 47, 50, 52, and 57 kD. The incidence of antibodies to these antigens in AA was up to seven times more frequent than in control sera and their level up to 13 times greater. The 44–52-kD antigens reacting with AA antibodies were HF specific. All were expressed in HF extracts, but could not be detected in similar extracts of adjacent scalp epidermis or dermis obtained from the same individual or other control tissues. By immunofluorescence some AA patients react with HF but not with adjacent epidermis or dermis confirming the presence of HF-specific antibodies in AA.

These differences were statistically significant for all five antigens. The nature of the HF antibody response in AA was further examined by analyzing the immunoglobulin isotype usage in AA and control persons. The anti-HF antibodies in control persons were present in low titer and were both IgM and IgG antibodies, whereas in AA the HF antibodies were high titer and predominantly IgG.

More recently, we have conducted studies to identify the subset(s) of cells in HF that are the target(s) of immune responses in AA. We have developed methods to culture individually the major HF cell subpopulations including, for the first time, HF melanocytes [4]. We believe it is critical to use cells derived from HFs rather than cells of similar histologic type derived from epidermis to study immune abnormalities associated with AA, based on evidence that similar cells derived from these two sites are immunologically different. As a result, the use of cells derived from epidermis as targets in immune assays may fail to reveal immune responses directed specifically to cells of HF.

Third, using isolated subsets of HF cells as targets, we found that AA antibodies appear to be directed to both keratinocytes and melanocytes in HF. In these studies we studied the ability of antibodies in AA and control individuals to react to pure populations of keratinocytes and melanocytes derived from HF. Antibodies to HF keratinocytes, as-

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